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Phage adsorption on Enteropathogenic and Shiga Toxin-Producing *Escherichia coli* strains: Influence of physicochemical and physiological factors

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ABSTRACT

Bacteriophages have proved to be useful tools against pathogenic *Escherichia coli* strains. The first step in the phage life cycle is the adsorption to the host cell surface. In the present work, three *Myoviridae* phages (DT1, DT5 and DT6) were used to characterize the adsorption process on three pathogenic *E. coli* strains, namely two Shiga-toxin producing *E. coli* (STEC) and one enteropathogenic *E. coli* (EPEC), in several conditions found on food. The influence of Na^{+}, Mg^{2+}, temperature, pH, periodate, proteinase K and physiological cell state on phage adsorption was investigated. The three phages evaluated showed high adsorption rates at pH 7.5 and 5.7 while they were moderate at the lowest pH evaluated (4.5). Sodium or magnesium ions were not indispensable for the adsorption of the three phages evaluated. Specifically, phage particles were adsorbed either in the presence or absence of Mg^{2+}, while increasing Na^{+} concentration resulted in lower adsorption values for all the systems evaluated. Regarding temperature, phage adsorption was slightly affected at 4 °C and 50 °C, while it reached its maximum at 37 °C. Adsorption rates decreased after the thermal inactivation of cells, though, when chloramphenicol (as protein-synthesis inhibitor) was used, adsorption values on treated and untreated cells were similar. In addition, periodate was able to decrease phage adsorption, thus suggesting the receptors were carbohydrates in nature. All these results showed that the adsorption process was only partially affected and most conditions are suitable for the completion of the first step in the phage life cycle. Therefore, phages evaluated in this study can be used to prevent foodborne diseases on several food matrices since they are active in a wide range of environmental conditions.

1. Introduction

The incidence of foodborne pathogens such as *Escherichia coli* continues to increase considerably in many countries (Farrokh et al., 2013; Rivas et al., 2008). Pathogenic *E. coli* strains are classified into six virotypes according to their mechanism of action: enteropathogenic (EPEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), diffuse adherence (DAEC) and enterohemorrhagic (EHEC). Enterohemorrhagic *Escherichia coli*, including STEC strains, are human pathogens that can cause diarrhea as well as hemorrhagic enterocolitis and hemolytic uremic syndrome (HUS) in humans (Griffin, Mead, & Sivapalasingam, 2002; Yoon & Hovde, 2008). Also, EPEC strains are responsible for human outbreaks worldwide (Costin, Voiculescu, & Gorcea, 1964; Varela et al., 2007; Viljanen et al., 1990), thus both foodborne pathogens, STEC and EPEC, represent a serious health threat.

Bacteriophages are the natural enemies of bacteria and they have received renewed attention in recent decades for potential applications as biocontrol agents (Storms, Smith, Sauvageau, & Cooper, 2012). The first step in the phage life cycle is the adsorption on the host cell surface, being a necessary step in almost all phage applications. The recognition of different cell receptors, as well as the variable length of the lipopolysaccharide (LPS) O chain, might be cited as the main variables when considering the adsorption process on *E. coli* strains. Pathogenic strains generally have LPS of greater length than non-pathogenic strains, making them less accessible to phage infection. In addition, *E. coli* strains may present a differential production, either a decrease or an alteration of phage receptors, which leads to a modified adsorption behavior. Therefore, knowledge of phage-host interactions becomes essential for the successful application of phage treatments. Phage-host interactions occur by a highly specific attachment between adhesins on the phage tail fibers and specific cell receptors (Goldberg, Grinius, & Letellier, 1994), though adsorption also depends on environmental factors (Hadas, Einav, Fishov, & Zaritsky, 1997). Even though many phage-

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host adsorption studies were previously performed, those focusing on EPEC and STEC strains have been scarcely documented. This is because most trials were conducted on laboratory strains such as *E. coli* F (Heller & Braun, 1979), B and K-12 (Guosheng et al., 2003; Hadas et al., 1997; Moldovan, Chapman-McQuiston, & Wu, 2007; Storms et al., 2012) rather than on pathogenic strains.

In previous studies, three coliphages, named DT1, DT5 and DT6 were demonstrated as efficient biocontrol agents of EPEC and STEC strains in meat (Tomat, Migliore, Aquili, Quiberoni, & Balague, 2013a) and milk matrix (Tomat, Mercanti, Balague, & Quiberoni, 2013b), as well as surface decontaminants (Tomat, Quiberoni, Mercanti, & Balague, 2014). Thus, the aim of the present work was to evaluate the influence of physicochemical and physiological parameters on phage adsorption and to characterize the interaction of those three coliphages with each pathogenic host strain in order to maximize adsorption efficiency on food environments.

2. Materials and methods

2.1. Bacterial strains and phages

E. coli DH5α was used as a sensitive strain to propagate all the bacteriophages assayed in this study. Three additional strains were used in the experiments. Two of them, an *E. coli* enteropathogenic (EPEC920) (*eae*) and an *E. coli* Shiga toxigenic O157:H7 (STEC464) (*stx2* and *eae*), were isolated from stool samples, identified using API-20E system (Biomerieux, Buenos Aires, Argentina), and further characterized by PCR. The third strain was an *E. coli* Shiga toxigenic non-O157:H7 (ARG4827; serogroup O18; *stx1* and *stx2*) (Balague et al., 2006). The *E. coli* strains were routinely reactivated overnight (37 °C) in Hershey broth (8 g l^{-1} Bacto nutrient broth, 5 g l^{-1} Bacto peptone and 1 g l^{-1} glucose) (Difco, Detroit, Michigan, USA) supplemented with $MgSO_4$ (5 mmol l^{-1}) (Cicarelli, San Lorenzo, Santa Fe, Argentina) (Hershey-Mg). All the strains were maintained as frozen (−80 °C) stock cultures in Hershey broth supplemented with 15% (v/v) glycerol.

Bacteriophages DT1, DT5 and DT6 were isolated from stool samples of patients with diarrhea treated at the Centenary Hospital, Rosario (Tomat, Mercanti, Balague, & Quiberoni, 2013b). Phage DT6 is active against the three strains evaluated whereas DT1 only against two, namely EPEC920 and O157:H7 STEC464. For this reason DT5 replaced DT1 in assays with non-O157 STEC ARG4827 strain. High-titre phage suspensions were prepared as previously described (Tomat, Mercanti, Balague, & Quiberoni, 2013b). Phage stocks were stored at 4 °C and enumerated (plaque formation units per millilitre; PFU ml^{-1}) by the double-layer plaque technique (Jamalludeen et al., 2007).

2.2. Adsorption studies

E. coli strains were grown overnight in Hershey broth at 37 °C. Adsorption was performed by mixing 100 μl of phage, ca. 10^5 PFU, with 500 μl of bacteria ($OD_{630nm} = 1$) at a multiplicity of infection (m.o.i) of ~0.001. The suspension was incubated at 37 °C for 10 min and centrifuged at 14,000 ×g for 5 min, and the phage titer remaining in the supernatant (free phage) following phage-host interaction was determined by the double-layer plaque technique (Tomat, Mercanti, Balague, & Quiberoni, 2013b). Nonadsorbing control was performed in each assay (Hershey broth) for setting the 100% free-phage titer.

2.2.1. Influence of cations

The influence of Na^+ and Mg^{2+} on phage adsorption was investigated by incubation (37 °C) of infected *E. coli* cultures in Hershey broth, with and without NaCl (0.3%, 2% and 4%) or $MgSO_4$ (1, 5 and 10 mmol l^{-1}). After 10 min, adsorption was determined as described above and the counts were compared with the titre of a control without cells at each ion concentration assayed. The results were expressed as percentages of phage adsorbed.

2.2.2. Influence of temperature

The adsorption of phages on *E. coli* cells was determined in Hershey-Mg broth at 4, 37 and 50 °C. Results were expressed as percentages of adsorption after 10 min of phage-cells interaction regarding non-adsorbing controls at each temperature evaluated.

2.2.3. Influence of pH

The adsorption of phages on *E. coli* cells was determined in Hershey-Mg broth at pH values of 4.5, 5.7 and 7.5. Results were expressed as percentages of adsorption after 10 min of phage-cells interaction regarding non-adsorbing controls at each pH evaluated.

2.2.4. Influence of thermal treatment on cells

Adsorption was determined in Hershey-Mg broth both on viable and nonviable cells as described above. Nonviable cells were obtained by keeping an *E. coli* cell suspension in boiling water for 1 min. Nonviability (100% of cell death) of treated cells was checked by plate counts.

2.2.5. Influence of cell protein-synthesis inhibitor

The minimum concentration of chloramphenicol (Calbiochem, California, USA) needed to inhibit the protein-synthesis on *E. coli* strains evaluated in this study was determined as follows. Exponentially growing ($OD_{630nm} = 1$) cell cultures in Hershey-Mg broth were centrifuged and cells resuspended (~1×10^8 CFU ml^{-1}) in Hershey-Mg broth with 38 μg ml^{-1} or 153 μg ml^{-1} of chloramphenicol and then incubated at 37 °C (during 240 min) after distributing into Eppendorf tubes. A cell culture subjected to a similar treatment but without chloramphenicol was used as control. At regular intervals, OD_{630nm} was measured and cell counts were assayed. The concentration of chloramphenicol selected to perform the adsorption experiment was the lowest producing stable values in the OD_{630nm} and plate counts during the incubation time. The inhibition of protein-synthesis was achieved after 90 min of incubation.

Additionally, it was also evaluated whether the effects of the protein-synthesis inhibitor were maintained after the removal of chloramphenicol. A culture with the same treatment described above was incubated at 37 °C to inhibit protein-synthesis (90 min). Then, cells were centrifuged, washed and resuspended in Hershey-Mg broth, and finally incubated at 37 °C. At regular intervals, OD_{630nm} was measured.

On the basis of the preceding experiments, phage adsorption assays were performed on host cells treated with chloramphenicol (38 μg ml^{-1}) that was removed after the inhibition of protein synthesis was achieved. Treated cells were infected with each phage (m.o.i ~ 0.001) and then incubated at 37 °C during 10 min. After centrifugation (14,000 g for 5 min), the titres of unadsorbed free phages in the supernatants were assayed as indicated, and the results expressed as percentages of the adsorption. A cell culture subjected to a similar treatment but without chloramphenicol was used as adsorption control.

2.2.6. Influence of periodate and proteinase K

Additional tests were performed to examine whether periodate and proteinase K are able to destroy the phage receptor, thus affecting phage adsorption to bacterial cell. In order to test the effect of periodate on phage adsorption, 1 ml of each strain culture was centrifuged at 14,000 × g for 5 min, and the bacterial pellet was suspended into 1 ml sodium acetate (50 mM; pH 5.2) or sodium acetate containing 1 mM $NaIO_4$. The cells were incubated for 2 h (protected from light), washed three times with 1 ml Hershey, centrifuged, and suspended in Hershey. Regarding proteinase K, 1 ml of each strain culture was centrifuged and treated with 0.2 mg ml^{-1} (1 ml Buffer; 50 mM Tris-HCl pH 8.0 and 10 mM $CaCl_2$) (Promega, Madison, USA) at 37 °C for 3 h and washed three times with 1 ml of Hershey. Phage adsorption assays after periodate and proteinase K treatments were performed as described above.

2.3. Statistical analysis

All data were analyzed using the Statgraphics™ Plus software (v 3.0, Statistical Graphics Corp.). Assays were carried out in triplicate. Means were compared using the one-way ANOVA procedure followed by Duncan's multiple range tests at $p < 0.05$.

3. Results and discussion

3.1. Adsorption studies

3.1.1. Influence of cations

Several foods, especially meat products, possess variable concentrations of salt reaching 4% or higher in some products such as cold cuts. For this reason, phage adsorption in the presence of monovalent ions such as Na^+ as well as those potentially involved in the adsorption process, namely divalent ions such as Mg^{2+}, was evaluated. The results showed that neither Na^+ nor Mg^{2+} was indispensable for adsorption of the three phages studied. Phage adsorption in Hershey broth was significant even without the addition of Mg^{2+} for all phage/strains systems evaluated (Fig. 1). In the absence or presence of Mg^{2+}, adsorption values ranged from 93.93% to 97.72%. In addition, the highest Mg^{2+} concentration assayed showed no effect on phage adsorption. In contrast, sodium ions have a significant ($p < 0.05$) influence on phage adsorption, especially at the highest Na^+ concentration which produced only slight decreases on this process. After 10 min, high adsorption values, namely up to 95.4%, were observed at 0.3% of Na^+ while at increased Na^+ concentrations a lower adsorption, ranging from 59.1% to 89.1%, was observed in all systems evaluated (Fig. 2). Although previous studies showed that higher concentrations of Na^+ resulted in reduced lytic activity of the three phages evaluated (namely, DT1/EPEC = 58.8%, DT1/ O157:H7 STEC = 61.6%, DT5/non-O157 STEC = 56.5%, DT/EPEC = 60.8%, DT6/non-O157 STEC = 55.2%, DT6/O157:H7 STEC = 72.0%; unpublished results) in proportions similar to the decrease in adsorption observed in this study, cell and phage viability remain unchanged for all the ion concentrations assayed. A possible explanation of this reduced adsorption is that addition of monovalent ions such as Na^+ may affect phage adsorption by displacing cations such as Ca^{2+} leading to destabilization of lipopolysaccharide layers, thus interfering with phage adsorption. An example of this is evidenced in the DT6/O157:H7 STEC464 system, where O157:H7 STEC464 is more affected by the higher concentration of Na^+ (4%) than the other strains tested, leading to the lowest phage adsorption value observed. However, results found indicate a low need for and a high tolerance to these cations, which would allow the use of these phages in food environments. For other coliphages, the requirement for Na^+ and Mg^{2+} for the adsorption process varied depending on the surface evaluated (Farrah, 1982; Puck, Garen, & Cline, 1951; Schwartz, 1976).

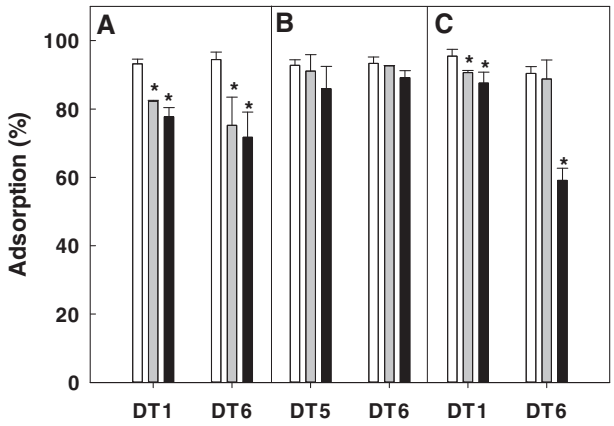

Fig. 2. Phage adsorption in Hershey broth at 37 °C with 0.3% ( ), 2% ( ) and 4% of Na^+ ( ) on EPEC920 (A), non-O157 STEC ARG4827 (B) and O157:H7 STEC464 (C) viable cells. Error bars represent the standard deviation of three determinations. Columns with asterisk are significantly different ($p < 0.05$) from the corresponding control (first column).

3.1.2. Influence of temperature

Foodstuffs are subjected to different temperatures during manufacture and storage; therefore, adsorption and viability of phages at refrigeration and abusive temperatures were evaluated. The three phages evaluated remained approx. 99% viable after exposure to low and high temperatures, 4 and 50 °C respectively, which is in agreement with the findings of Coffey et al. (2011) that phages of *E. coli* O157:H7 remain viable after exposure to a wide range of temperatures, namely between −22 and 60 °C. In addition, viability of the three *E. coli* strains tested was not affected by high temperatures, namely 50 °C. The effect of temperature on phage adsorption is shown in Fig. 3. As expected, phage adsorption tested in the present study was maximum at 37 °C whereas it was slightly affected at 4 °C and 50 °C. Likewise, similar results were found for coliphages such as λ at 4 and 40 °C (Moldovan et al., 2007). Phages DT1 and DT5 showed the lowest adsorption values (approx. 91.0%) at 4 °C while DT6 at 50 °C on the three strains tested (88.5%, 92.9% and 83.2% for EPEC, non-O157 and O157:H7 STEC strains, respectively), however, relatively high values were obtained at both conditions. Reductions observed at 50 °C may be due to disorganization

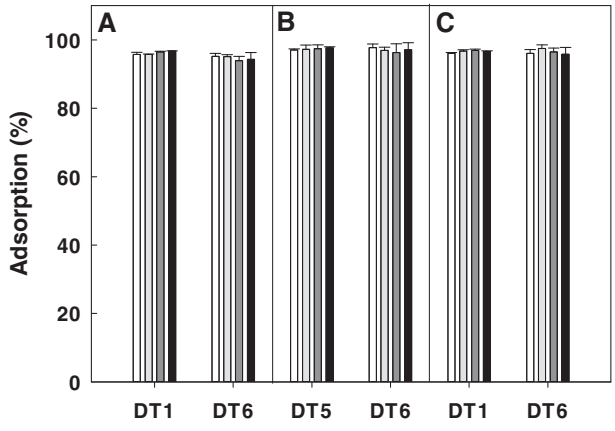

Fig. 1. Phage adsorption in Hershey broth at 37 °C without ( ) and with 1 mM ( ), 5 mM ( ) and 10 mM of Mg^{2+} ( ) on EPEC920 (A), non-O157 STEC ARG4827 (B) and O157:H7 STEC464 (C) viable cells. Error bars represent the standard deviation of three determinations.

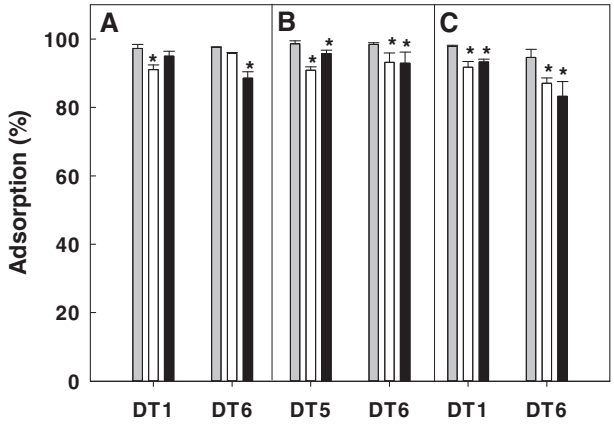

Fig. 3. Phage adsorption in Hershey broth at 37 °C ( ), 4 °C ( ) and 50 °C ( ) on EPEC920 (A), non-O157 STEC ARG4827 (B) and O157:H7 STEC464 (C) viable cells. Error bars represent the standard deviation of three determinations. Columns with asterisk are significantly different ($p < 0.05$) from the corresponding control (first column).

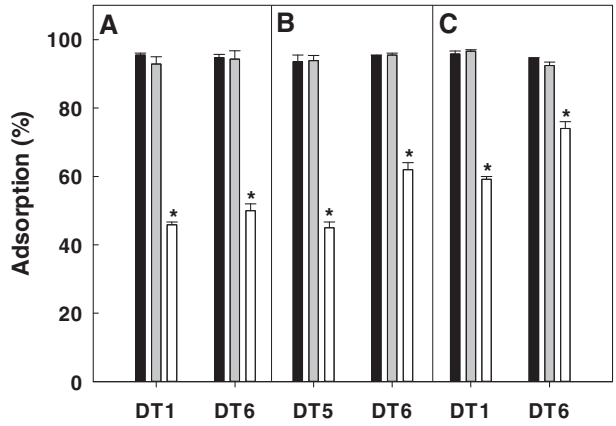

Fig. 4. Phage adsorption in Hershey broth at 37 °C and pH of 7.5 (■), 5.7 (▒) and 4.5 ( ) on EPEC920 (A), non-O157 STEC ARG4827 (B) and O157:H7 STEC464 (C) viable cells. Error bars represent the standard deviation of three determinations. Columns with asterisk are significantly different ($p < 0.05$) from the corresponding control (first column).

and/or partial denaturation of phage receptors on the bacterial cell surface as a result of the high temperature, thus hindering phage adsorption. Furthermore, high temperatures may modify the structure and function of bacteriophages (Coffey et al., 2011). At 4 °C, adsorption values ranged from 87.1% to 95.8%. As mentioned, adsorption values obtained at low temperature were lower than at 37 °C although still considerably high. Kudva et al. (1999) proposed that low temperatures and the absence of bacterial growth may favor the phage adsorption process, though greater adsorption is observed as the temperature approaches the optimum growth temperature for the host bacterium (Briggiler Marcó, Reinheimer, & Quiberoni, 2010; Moldovan et al., 2007). These observations are in accordance with the results obtained in the present study for all phage/strain systems.

3.1.3. Influence of pH

Phages selected for food safety, especially for acidic foodstuffs, must be resistant and withstand low pH values in order to maintain their viability on the food matrix and reach their target cell at an adequate concentration. Meat and dairy products have characteristic physicochemical parameters. For instance, after slaughtering, meat pH falls to 5.5–6.0 while several dairy products reach values as low as 4–4.5. Our results indicated that phage viability was affected at the lowest pH value assayed, thus, phage viability losses were taken into account by calculating percentages regarding non-adsorbing controls at each pH evaluated. The three phages in this study exhibited high adsorption values, up to 96.5%, at pH 7.5 and 5.7 on the three *E. coli* strains used whereas at pH 4.5, phage adsorption was lower and ranged from 45.0 (phages DT1 and DT5) to 74.0% (phage DT6), though moderate values were observed (Fig. 4). On the contrary to that observed at high monovalent ion (sodium) concentration with the DT6/O157:H7 STEC464 system, the lowest pH -high H^+ concentration- assayed slightly affected phage adsorption. In contrast with results obtained for EPEC strain, higher stability of receptors present in O157:H7 STEC464 strain at low pH values may be related to different length and/or composition of LPS. Jamalludeen et al. (2007) found high phage resistance up to pH 5, while both viability and lytic activity were affected at a lower pH value. In addition, when *E. coli* phage infectivity below pH 3 was evaluated, no viable particles were found (Dini & de Urraza, 2010). According to the aforementioned, low pH values are expected to affect the phage adsorption process by changes in the acid-base status leading to conformational modifications of the phage and/or of their cell surface receptors. In addition, although viability controls were carried out at each pH tested, the wide range found for phage adsorption at pH 4.5 may be due to a differential loss of phage viability in the presence and absence of bacterial cells.

3.1.4. Influence of thermal treatment and protein-synthesis inhibitor on cells

The ability of thermally inactivated cells to adsorb phages was also evaluated. For all the systems studied, when cell suspensions were inactivated by heat, adsorption of phages was significantly different ($p < 0.05$) with respect to those obtained on viable cells. After 10 min, adsorption on nonviable cells ranged between 37.6% and 52.1%, depending on the phage, while adsorption on viable cells was significantly higher for all phage/strain systems evaluated (Table 1). The diminished phage adsorption observed on thermally killed cells could be linked to modifications on membrane permeability leading to the loss of electrolytes and proton motive force as well as diminished ATP content. The above mentioned factors may also lead to conformational changes, reduced affinity and altered spatial distribution of phage receptors on bacterial cell surface (Bayer, 1968; Zgaga, Medic, Salaj-Smic, Novak, & Wrischer, 1973). Heller and Braun (1979) found that *E. coli* F cells with an altered receptor structure exhibited low adsorption values of the phages studied.

Phage adsorption on the host strains subjected to protein synthesis inhibition was tested in order to evaluate whether changes on membrane permeability and low ATP content could modify phage adsorption. The lack of bacterial cell energy and/or disorganization of phage receptors sites on thermally inactivated cells could be factors affecting phage adsorption. Therefore, protein synthesis inhibition experiments, unlike high temperatures which produce disorganization of receptors and loss of cell integrity, allow us to discriminate if these factors are involved in the adsorption process. The chloramphenicol concentration used in the experience was 38 μg ml^{-1}; under that condition, inhibition of protein-synthesis on cells was maintained after the removal of chloramphenicol. Therefore, the phage adsorption process was carried out in the absence of antibiotic. Treatment with chloramphenicol on cells did not influence the adsorption values ($p > 0.05$) for all systems evaluated when they were compared with those attained with untreated cells. Adsorption values on cells treated with chloramphenicol ranged from 94.8% to 99.0% (Table 1). In addition, results with chloramphenicol indicate that protein synthesis is not obligatory for the adsorption process. Authors such as Hancock and Braun (1976) and Daugelavicius, Bamford, and Bamford (1997a) found that phage adsorption is an energy-dependent process. Furthermore, several *Escherichia coli* T-series phages adsorb irreversibly only to host cells that have a high transmembrane proton motrice force (Daugelavicius, Bamford, Grahn,

Table 1
Adsorption rates (Hershey, 37 °C, 10 min) of DT1, DT5 and DT6 phages on protein-synthesis inhibited and thermally inactivated *E. coli* cells.

Treatment	* Phage Adsorption (%) [a] on *E. coli strains*					
	EPEC920		non-O157 STEC		O157:H7 STEC	
	DT1	DT6	DT5	DT6	DT1	DT6
Control without treatment	97.8 ± 0.3	95.2 ± 0.3	97.3 ± 1.2	94.3 ± 1.7	98.7 ± 0.1	95.8 ± 2.8
Thermally inactivated cells	43.0 ± 1.1	50.2 ± 2.5	50.8 ± 1.1	52.1 ± 0.7	37.6 ± 0.7	41.8 ± 1.2
Cells inhibited Cm (38 μg/ml)	98.5 ± 0.2	95.8 ± 1.1	98.3 ± 1.4	95.5 ± 1.3	99.0 ± 0.2	94.8 ± 4.2

*Percentage values regarding 100% non-adsorbing control. [a] Mean value of three determinations ± standard deviation. Cm: chloramphenicol.

Table 2
Adsorption rates (Hershey, 37 °C, 10 min) of DT1, DT5 and DT6 phages on *E. coli* cells treated with periodate and proteinase K.

Treatment	* Phage Adsorption (%) [a] on *E. coli strains*					
	EPEC920		non-O157 STEC		O157:H7 STEC	
	DT1	DT6	DT5	DT6	DT1	DT6
Control without treatment	94.8 ± 9.4	95.3 ± 7.7	91.6 ± 5.7	98.2 ± 7.4	89.5 ± 7.6	91.8 ± 8.8
Buffer without periodate	96.3 ± 3.5	92.5 ± 6.5	93.4 ± 8.6	82.4 ± 10.1	92.2 ± 3.8	96.7 ± 5.0
Periodate (1 mM)	13.8 ± 7.9	51.0 ± 5.3	0.0 ± 5.5	49.4 ± 8.0	20.7 ± 2.3	16.7 ± 9.0
Control without proteinase K	91.1 ± 7.1	92.7 ± 5.3	92.4 ± 8.9	89.3 ± 11.5	95.2 ± 10.9	93.3 ± 9.5
Proteinase K (0.2 mg/ml)	84.5 ± 3.7	87.0 ± 4.6	86.2 ± 4.3	88.8 ± 7.2	93.1 ± 6.0	86.7 ± 11.3

* Percentage values regarding 100% non-adsorbing control. [a] Mean value of three determinations ± standard deviation.

Lanka, & Bamford, 1997b; Keweloh & Bakker, 1984). Nevertheless, other authors obtained opposite results (Labedan & Goldberg, 1979; Yamamoto, Kanegasaki, & Yoshikawa, 1980). Despite the results obtained in the present study, further assays would be necessary to clarify results regarding the decreased adsorption observed on the thermally treated cells.

3.1.5. Influence of periodate and proteinase K

In the present work, receptor studies were performed on whole cells chemically — periodate — and enzymatically — proteinase K — treated. Cellular integrity of the chemically treated cells could be affected causing a subsequent release of intracellular substances that may interfere with adsorption experiments. For this reason, several post-treatment washing steps were performed prior to adsorption experiments. Phage adsorption on *E. coli* strains was significantly affected by periodate while treatment of bacterial cells with proteinase K produced minor reductions on phage adsorption (Table 2). Specifically, controls and treatments with proteinase K showed values ranging from 89.3% to 96.7% and 84.5% to 93.1%, respectively, while treatments with periodate produced an average adsorption value of approx. 25%. In addition, controls, namely buffer without periodate and control without proteinase K, were carried out and matrix influence on phage adsorption was discarded. Previous studies with phages DT1, DT5 and DT6 showed a low efficiency of plating (EOP) value with two pathogenic *E. coli* strains evaluated in this study, namely EPEC920 and ARG4827 (unpublished results). It is known that O antigens can participate both in the recognition of phage adhesins or interfering with the access to a receptor located more internally, thus reducing phage adsorption (Kutter, Raya, & Carlson, 2005), especially on long length lipopolysaccharide (LPS) of pathogenic *E. coli* strains. According to adsorption results, factors other than LPS might be involved in the low EOP observed since, as indicated above, cells treated with periodate, which breaks down carbohydrates containing 1,2-diol motifs on their structure, showed a significant reduction on phage adsorption, thus the LPS would be involved in the recognition and adsorption of phages. In conclusion, treatments with periodate showed that receptors of *E. coli* phages evaluated possess carbohydrates on their structure. Similar results were found by other authors on O157:H7 *E. coli* (Tanji et al., 2004) and other bacterial species (Kiljunen et al., 2011; Sorensen et al., 2011).

On the other hand, although periodate assays evidenced carbohydrates on phage receptors, treatment with proteinase K also produced minor reductions in the adsorption rate. Phage receptors of proteinaceous nature have also been described in *E. coli*. These receptors are located on the external membrane and are generally porins such as OmpC (Yu, Ko, Chen, & Syu, 2000), transport or pili proteins (Rakhuba, Kolomiets, Dey, & Novik, 2010). Moreover, as known, proteins are denatured by heat, disrupting and affecting the availability of phage attachment sites and leading to low adsorption rates, as observed in this study. Thus, based on the results attained in the receptor assays and the adsorption observed on thermally inactivated cells, we hypothesize that there might be proteins acting as co-receptors involved in the phage adsorption process.

4. Conclusion

In spite of the relevance of *E. coli* in foodborne diseases, very limited information is available in studies regarding phage adsorption on pathogenic *E. coli* strains. As stated previously, all the preceding research conducted on this subject on *E. coli* was specifically focused on laboratory *Escherichia coli* strains such as *E. coli* B, F and K-12, and, to our knowledge, this is the first study focused on EPEC, non-O157 STEC and O157:H7 STEC strains. A better understanding of the conditions affecting phage-cell interactions would make it possible to develop strategies to increase the killing of *E. coli* strains in food environments. Data obtained in this work have demonstrated the influence of physicochemical and physiological factors on the adsorption of three *Myoviridae* phages on pathogenic *E. coli* strains. Considering that phage adsorption was high in most of the conditions that emulate those found on foods, we conclude that the phages studied in this work are useful for biocontrol purposes in food environments. Although a carbohydrate nature was suggested for the phage receptors studied here, further research is needed to specifically identify cell receptor components for each phage.

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